THE OCCURRENCE OF BRUCELLA ORGANISMS IN THE MILK OF BOTH VACCINATED AND UNVACCINATED CATTLE

BY

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INTRODUCTION

Brucellosis is a disease of animals and man caused by three species of the genus Brucella. These species are Brucella abortus (the bovine type) causing a disease in cattle commonly referred to as contagious abortion; Brucella melitensis (the caprine type) causing a disease in goats and man known as Malta fever; and Brucella suis (the porcine type) causing a disease in hogs commonly referred to as contagious abortion of swine. The three species are very closely related and all three cause a disease in man known as undulant fever. Brucellosis is primarily a disease of the lower animals; communicable to man, but probably never or rarely communicated from man to man. Man is infected directly through the skin by contact with diseased animals or their tissues and discharges; or indirectly, through the consumption of raw milk and milk derivatives.

A major problem facing the farmer, the dairy man, and the public health official is the occurrence of the organism causing brucellosis in the milk of dairy cattle.

Many studies have been made of brucellosis in cattle caused by Brucella abortus and its relation to man. Due to these studies, various precautions have been taken to prevent the spread of brucellosis from animals to man. The disease is known to rank high in its dire effects upon men in many occupations. The farmer, the dairy man, the meat handler, the consumer of unpasteurized milk, and the veterinarian all have been affected.

Sanitary methods used in the milking processes together with the major defense, pasteurization, prevent the spread of the disease by means of milk consumption.

The vaccination of cattle at calfhood with Bureau of Animal Industry (B.A.I.) strain 19 Brucella abortus is being practiced. B.A.I. strain 19 Brucella abortus is an avirulent strain which is thought to build up an increased resistance in the animal against the entrance of the virulent form of Brucella abortus. The prevention of this disease in cattle thereby prevents the transmission to man.

In view of the widespread interest in the control of this disease, it seems a study of the milk of dairy cattle vaccinated with B.A.I. strain 19 of Brucella abortus and the milk of cattle not vaccinated would be of interest. Such a study would determine the occurrence of the brucella organisms. This determination could be incorporated in the control measures taken to prevent the disease of brucellosis.

The characteristics of the brucella organism in milk make definite cultural procedures necessary. The investigation was carried out in two parts: (1) a laboratory study to detect the presence of brucella organisms in milk; (2) a laboratory study to identify and differentiate Brucella abortus from the other brucella organisms found in milk.

REVIEW OF LITERATURE

The disease of Brucellosis under many different names has been known for centuries, but was not well understood until less than half a century ago when the causative agent was isolated by Bruce. Hippocrates described a disease in man characterized by protracted fever with a tendency to relapse, which somewhat resembled tuberculosis and which was not fatal. This disease was probably undulant fever. Nothing more was recorded until Burnett (1816) described the disease under the name of remittent "malarial fever".

The first record of a Brucella infection was reported by Marston in 1859. He called the disease "Mediterranean" or "gastric" fever. Bruce in 1886 isolated the organism causing the fever from the spleen of a victim of the disease, and later named it Micrococcus melitensis. The first two reported infections were due to the goat strain and were referred to as "Malta" fever because their source was goats on the island of Malta.

Because of the prevalence of the fever among the British soldiers and sailors stationed on the island of Malta, the British government in 1904 appointed a commission to investigate the sources of infection and advise methods of control. As early as 1905, the British Commission for the Investigation of Mediterranean Fever in 1905-1907 connected the disease with goats and gave the first accurate knowledge of its epidemiology. Six reports were issued by the commission. Subsequent studies indi-

cated that goats were easily infected; that about 50 percent of them acquired the disease naturally; and that the causative organism was eliminated in the milk and urine. Ten percent of the goats in Malta were found to be eliminating the organism in milk; moreover, this was highly infectious for monkeys, in which case it produced attacks similar to the fever in man.

Contagious abortion in cattle has a parallel record of antiquity. The disease has been known in Europe for centuries where its infectiousness, together with the losses caused by it, are recorded by Moscal in 1567, Lawrence in 1805, and Skellet in 1808. Jonati in 1837 and Barlow in 1851 also added to the knowledge of the disease.

St. Cyr in 1875 stated his belief in a specific agent, and in 1876 Franck communicated the disease to healthy cattle by introducing the discharge and fetal membranes from aborting animals. Lehnert in 1878 and Brauner in 1880 corroborated this work. Nocard in 1888 isolated two organisms, a short bacillus and a micrococcus, but failed to show reproduction of the disease in healthy animals.

Ten years after Bruce isolated the causative agent of Malta fever, Bang, assisted by Stribolt in 1896, discovered the organism causing the disease in cattle. He ascribed it to an organism which produced the disease on artificial inoculation, and which could be recovered from the infected animals. The disease could be reproduced at will with pure cultures of this organism. The disease is now designated as Brucellosis; the organism as Br. abortus. The characteristics of Bang's disease are inflam-

matory changes of the mucous membrane of the uterus and fetal membranes causing the premature expulsion of the fetus. A chronic interstitial mastititis is produced in the udder of the cow.

The exact date at which Brucellosis was introduced into the United States is not known. The most probable assumption is that it was brought in quite early in the history of the colonies with the introduction of cattle. The discovery that the bacillus of Bang is excreted in milk seemed to offer a satisfactory explanation of the brucellosis of man in those parts of the United States and other countries that do not have a large milk goat industry.

Various investigators have observed that guinea pigs, inoculated with cow's milk for the purpose of detecting M. tuberculosis and autopsied after extended periods, showed lesions which could easily be mistaken for tuberculosis but in which no acid-fast organism could be demonstrated. Schroeder and Cotton (1911) noted this condition in a series of tests and found it transmissible from one guinea pig to another by subcutaneous inoculation of affected tissue. The responsible organism was eventually cultivated and its similarity to the organism of contagious abortion led Mohler and Traum (1911) to suspect that the two were identical.

Evans (1915) found that Bang's bacillus resembled in cultural and serological characteristics the micrococcus discovered by Bruce. She remarked in her report that since Bruce's organism caused Malta fever in man, one might logically expect to find

similar infections from the bovine strain.

Traum (1914) noted a similar infection in swine. The pig or porcine strain differs in some characteristics and is now called the Brucella suis.

There are numerous references in the literature to the use of bacterins or killed suspensions to combat brucellosis, but none has proved effective. Giltner, Hallman, and Coolidge (1916) reported discouraging results with their experiments. Huhtala (1931) of Finland had similar results with biological preparations that others of both America and Europe experienced.

Many investigators have attempted to control "contagious abortion" through the use of both killed and living Br. abortus vaccines. Bang (1906) utilized killed bacteria for the prevention of the disease. The results obtained were not positive.

Huddleson (1934) states:

> One of the chief fallacies in the use of killed and living vaccines that many investigators were thinking of the symptoms of a disease, that is, premature expulsion of the fetus, rather than of the prevention of infection. So for many years the idea behind the employment of vaccines was to prevent abortion rather than to prevent infection. It has only been in recent years that the nature of the disease in the cow has been fully understood. It is now known that prevention of infection is just as important, if not more so, than the prevention of the symptoms of the disease. It is known that infection in the udder, which occurs in an infected animal, is just as important economically as the loss of the calf by premature expulsion.
>
> Failure to understand the nature of the disease in past years led to the injection of living, virulent cultures into non-pregnant animals. After inoculation, it was observed that a large percentage of the animals so treated carried their calves to maturity, even when they had become infected. As the result of early work of this nature, promiscuous injection of cattle with virulent cultures took place throughout the United States and various other countries. Many manufacturers

> biologicals even went so far as to advertise the fact that great pains were taken to keep the organism used in the vaccine alive and virulent. For a time not only was Br. abortus used in the preparation of the vaccine, but certain individuals and manufacturers of biologicals resorted to the use of living cultures of Br. suis. There is no doubt that as a result of the wide use of virulent cultures in the preparation of vaccines thousands of cattle became infected. This served to increase the incidence of infection in dairy cattle in European countries as well as in the United States.

He says that most of the animals that become infected naturally are nonpregnant and it would be reasonable to expect the same results with artificially infected animals.

Huddleson (1943) in his early studies of 1921-1925 says that experiments showed that a vaccine prepared from dissociated cultures of Brucella failed to show any appreciable immunity in susceptible animals.

Smith and Little in 1923 were among the first to study vaccination in this country. They stated (1923) that infection may be eradicated by the destruction of all infected animals, or resistance can be increased through the use of vaccination. They used two live virulent cultures as vaccines and were able to show marked improvement in the lowering of abortion rate.

The experiments of Buck and Creech (1924) indicated that the inoculation of nonpregnant heifers of breeding age with live cultures of Br. abortus seldom gave permanent infection. Hart and Traum (1925) also used virulent cultures in their vaccination studies of immunity in animals. They demonstrated the immunogenic properties, also the power of the organisms to establish themselves in the udder of the lactating cow.

Buck in 1925 experimented with living Br. abortus sus-

pensions. He endeavored to show the efficacy of suspensions to produce enough immunity in calves for protection against infection when they became mature. Three calves treated with No. 19 resisted infection on artificial exposure. Carpenter (1926) experimented with living virulent cultures of Brucella showing cattle vaccinated gave udder infections in a high percentage of cases.

Cotton and Buck (1931) tested vaccines of different degrees of virulence and stated that highly virulent strains are objectionable because of the danger of implanting the organism in the udder. Vaccines of lessened virulence gave satisfactory results toward prevention of abortion and udder infection.

Cotton and Buck (1932) made a subsequent report on their vaccination studies as follows:

> We are led to suspect strongly that, by selecting Br. abortus strains of proper virulence for vaccine preparation and by confining the use of vaccine largely to unbred animals, possibly calves or virgin heifers at near breeding age, immunization may be perfected to the point where in many herds it may be found to serve a useful purpose in reducing abortion losses and assisting herd owners gradually to eliminate the disease without at the same time being a menace to human health.

Cotton, Buck, and Smith (1934) concluded in continued experiments that calves between 4 and 6 months of age should be treated with B.A.I. strain 19 vaccine to avoid a prolonged serum agglutination titer.

In 1941 Hart and Traum collected data on the immunization value of B.A.I. strain 19 Br. abortus. They experimented with cows, heifers, and young calves. Over a period of 6 years 2,872 pregnancies occurred in 1,956 animals. There were 169

abortions. They estimated that 15 percent of these were due to brucellosis. Experiments with animals not infected were made to determine whether they would remain so while with infected animals. Their conclusions were that control by vaccination can be produced with proper herd management.

Rabstein and Welch (1941) in their studies of B.A.I. strain 19 vaccination reported:

> There was a direct relationship between the age of the animal and the time of vaccination and the length of time that a positive blood reaction was retained.
>
> Of the pregnancies recorded on animals vaccinated in this experiment, 172 have had one calf, 90 have had two, 48 have had three calves, 26 have had four, and 8 have had five calves each. Out of the total number of pregnancies, 10 or 1.5 percent terminated in abortions of which 5 appeared to be due to Br. abortus infection.
>
> After an average period of about four years under the calfhood vaccination plan, the total number of adult animals in the 11 experimental herds increased from 596 to 689. Three of the herds are now completely negative, having replaced their reactors with vaccinated animals of their own raising. Exclusive of their young stock, there are now 334 vaccinated animals, or 48.9 percent of the total number of these herds.

Rabstein and Welch also stated that some old reactors remained in the herds until unprofitable.

Haring (1942) reported the use of B.A.I. strain 19 Br. abortus in controlled experiments on cattle. It was used for the vaccination of calves and heifers on various farms. The report indicates that the vaccine proved to be useful in eradicating "Bang's disease" from badly infected dairy herds. A controlled test was made on a herd of 45 milk cows, 19 of which had agglutination titers of 1:100 or higher. Thirteen of these animals were shedding the Br. abortus organism in the milk. Infected

cows were not vaccinated and were removed from the herd when no longer profitable. There were no animals added to the herd from outside sources. The use of B.A.I. strain 19 Br. abortus vaccine on all heifers and calves was commenced September, 1933, and by July, 1939, the herd contained 68 cows, of which all except 8 were vaccinated as heifers. None of these animals gave a complete titer higher than 1:50 or an incomplete higher than 1:100. Br. abortus could not be revealed by repeated laboratory tests except in the case of one old cow which shedded the organisms until killed. Haring concluded that the disappearance of Br. abortus from the tested herd may be due to the recovery of infected animals; the removal of infected cows; and the effect of the vaccine in preventing the spread of infection to cows and young heifers.

Huddleson (1943) states that B.A.I. strain 19 as an immunizing agent should be used on calves between 4 and 8 months of age to develop enough immunity for protection during the first pregnancy.

McEwen (1937) in England reported investigations based on the use of strain 45 Br. abortus, a live culture of low virulence.

In conclusion the following statement of Huddleson (1943) seems appropriate:

> The proper and continued use of B.A.I. strain 19 should serve a useful purpose in preventing the spread of infection in infected herds and in preventing its occurrence in those free from brucellosis.
>
> It may play as useful a role in the control of brucellosis (Bang's disease) as the slaughtering of infected cattle.

Summary of results of vaccination with BAI Strain 19 as reported by several investigators.

	Method of exposure	Vaccinated		Controls	
		Total	Infected	Total	Infected
Cotton et al. (66)	Artificial	15	1(6.6%)	19	15(78%)
Hardenbergh (172)	Natural	143	3(2.4%)	73	4(6.2%)
Mills (311)	Natural	142	12(8.5%)	46	16(34%)
Thomsen (441)	Natural	266	9(3.3%)	135	134(25.5%)
Tompkins (443)	Natural	24	4(16.6%)	32	9(28.1%)
Tompkins (443)	Natural	222[a]	3(1.3%)	-	-
Birch et al. (27)	Natural	35[a]	10(28.5%)	23[a]	17(73.9%)
Mohler et al. (315)	Natural	8,182[b]	123(1.6%)	-	-
Haring and Traum (178)	Natural	2,872[c]	169(5.9%)[d]	1,763	245 (13.9%)[d]

a First parturition
b Report covers part of 3 parturitions
c Parturitions
d Abortions
e Spreaders and reactors.

Fig. 1. TABLE XXXVI (From Brucellosis In Man And Animals by Huddleson, I. Forest).

EXPERIMENTAL

Preliminary Methods and Procedures

Efforts to isolate Brucella organisms from the milk of infected cattle does not always give satisfactory results. For this reason, preliminary tests were made to develop a more satisfactory technique for the isolation of the organisms. Four known infected cows were used for preliminary examinations. Affirmative results were obtained from the tests to be named in subsequent work.

Collection of Milk Samples. The milk was collected at the afternoon milking time from the individual udders of the four animals selected and examined for the presence of organisms belonging to the genus Brucella.

The teats were wiped with a chlorine dampened cloth and the strip milk was discarded. Approximately 7.0 ml of milk was taken from each quarter and deposited into previously sterilized test tubes. The tubes were capped with heavy cotton plugs. A minimum time was utilized in the removal of the plugs to prevent contamination.

Preparation of Milk Samples. The milk samples were placed in the refrigerator immediately after the collection of the milk and allowed to cool and permit the cream to rise. The time for cooling varied between 18-24 hours.

Preparation of the Media. Bacto-tryptose agar was prepared in five liter quantities and used in the following recipe:

Bacto-tryptose	100 g
Bacto-dextrose	5 g
Sodium chloride	25 g
Bacto-agar	100 g
Distilled water	5 l

The tryptose agar media was bottled in 100 ml quantities and sterilized at 15 pounds steam pressure for 30 minutes after the pH was adjusted to the neutral point.

Because of the presence of Gram-positive contaminants in milk, crystal violet was introduced into the media to give a final concentration of 1 to 700,000 in the tryptose agar. This amount of dye is sufficient to suppress all Gram-positive organisms which might interfere. In order to secure the proper concentration of 1 to 700,000, 0.14 ml of crystal violet was added to 100 ml quantities of the media. The sterile medium was then poured into sterile petri plates and allowed to solidify.

Inoculation and Incubation. At the end of the cooling period, 0.1 and 0.2 ml quantities of gravity cream were deposited upon the surface of the medium by means of sterile pipettes. The plates were then rotated to allow the cream to spread over the surface of the medium. After all samples were plated, the plates were placed in anaerobe jars in an inverted position to incubate at 37 degrees centigrade. This jar contained an atmosphere of approximately 10 percent carbondioxide. The 10 percent carbondioxide atmosphere was attained by the use of a burning candle. The inoculated plates were incubated for a period of 5 to 7 days.

Agglutination Tests. Agglutination tests were made on the milk serum to determine the presence or absence of the Br. abortus agglutinating antibody. The gravity cream of the samples was

removed by means of sterile swabs. In some instances the pipette was used when excessive cream was present. This left the milk serum to which two drops of rennin for each 5 ml of milk was added. This was thoroughly mixed and incubated for two hours at 37 degrees centigrade in a slanting position to permit the curd and serum to separate. After incubation, the tubes were allowed to stand in a cool room 6 to 8 hours so as to obtain sera free from casein particles.

The materials used for the agglutination test were: (1) a glass plate ruled into squares; (2) 0.2 ml pipettes graduated in 0.01 ml; (3) clean toothpicks for mixing milk serum and antigen; (4) a properly standardized antigen and standard dropper pipette; (5) a box type lamp.

The serum samples were arranged in parallel rows with one in each square of the glass plate. The identification number was marked in wax pencil at the beginning of each row. The procedures used for placing the serum, mixing with the proper amounts of antigen, and interpretation of the test were taken from "Standard Methods for the Examination of Dairy Products".

Identification of the Brucella Organism. At the end of 5 to 7 days the petri plates were removed from the incubator and the colonies inspected for the presence of Brucella. Gram stains were made of all typical and suspicious looking colonies to determine their morphological characteristics. These colonies were then placed on tryptose agar slants and incubated under a 10 percent carbondioxide atmosphere for a period of five days. At the end of that period the cultures were removed and differentiation

tests were carried out.

All colonies developing on tryptose agar, containing 1 to 700,000 dilution of crystal violet, possessing any characteristics of Brucella were tested with known positive Br. abortus antiserum in a second rapid agglutination test. Any colony resembling a Brucella colony was tested.

The dye plate method, which makes use of the difference in growth characteristics of the species of Brucella in the presence of certain aniline dyes in a solid medium, was used. Thionin and basic fuchin were added to the solid medium to give final dilution each of 1:100,000. Organisms of each colony agglutinated by positive brucella antiserum were then inoculated on a plate of thionin and a plate of basic fuchin agar. An incubation period of 72 hours in 10 percent carbondioxide was used before determination as to species was made. Results of the dye test for Brucella species show that Br. melitensis and Br. suis will grow on tryptose agar containing thionin while Br. abortus is inhibited. Br. melitensis and Br. abortus develop on tryptose agar containing basic fuchin and Br. suis is inhibited.

Preliminary Results Obtained

Of the four cows selected for milk samples, one animal's left rear quarter milk sample was discarded due to a mastitis condition of the udder and milk sample. The results are based on individual quarter samples of the remaining cows.

Table 1. Rapid agglutination test of the milk serum.

Cow No.	Quarter[1]	Dilution[2] 1:25	1:50	1:100	1:200	1:400
137	R.F.	pos	neg	neg	neg	neg
	R.R.	pos	pos	neg	neg	neg
	L.F.	pos	neg	neg	neg	neg
	L.R.	pos	neg	neg	neg	neg
130	R.F.	pos	pos	pos	pos	neg
	R.R.	pos	pos	pos	pos	neg
	L.F.	pos	pos	pos	neg	neg
	L.R.	pos	pos	pos	pos	neg
450	R.F.	neg	neg	neg	neg	neg
	R.R.	?	?	neg	neg	neg
	L.F.	neg	neg	neg	neg	neg
	L.R.	?	?	neg	neg	neg
175	R.F.	pos	pos	pos	neg	neg
	R.R.	pos	pos	pos	pos	neg
	L.F.	neg	neg	neg	neg	neg
	L.R. (mastitis condition)					

[1] R.F. - right front quarter
R.R. - right rear quarter
L.F. - left front quarter
L.R. - left rear quarter

[2] pos. - a positive agglutination
neg. - a negative agglutination or no reaction
? - a questionable agglutination

Of the four animals tested, the milk serum of cow No. 130 gave an incomplete agglutination titer of no higher than 1:200; cow No. 175 an incomplete agglutination titer of no higher than 1:200; cow No. 137 an incomplete agglutination titer of 1:50; and cow No. 450 an incomplete questionable titer of 1:50. However, the agglutination tests made with suspensions of the slant cultured organisms agglutinated by positive antiserum revealed the presence of the Brucella organism from all cows except number 450.

Differentiation by the use of thionin and basic fuchin aniline dyes showed that 51 cultures of Brucella all grew on the basic fuchin tryptose agar medium but did not grow on the thionin tryptose agar medium. These cultures were developed from the milk samples.

Through these methods of isolation and identification it was determined that Brucella abortus organisms were present in the milk of cows numbers 130, 137, and 175. The tests did not reveal the presence of Brucella abortus organisms in the milk of cow number 450.

Methods and Procedures

Detection of the Presence of Brucella Organisms. Composite milk samples were obtained instead of the individual test samples.

The preparations of milk and media were identical with those in the preliminary examinations.

Inoculation and Incubation. Two methods were utilized to remove the cream and place it on the Petri plates; the pipette method and the swab method. The swab method was used on the later herds and found to be quite satisfactory.

Agglutination Tests. No variation in this test from the preliminary procedures was introduced.

Identification of the Brucella Organism. The milk samples of five herds, involving 118 vaccinated and 20 unvaccinated milk cows, were examined.

The milk samples of 118 vaccinated and 20 unvaccinated cows were found negative. Repeated tests failed to prove the presence of Brucella abortus organisms.

Results Obtained

The history of the herds is a result of questions answered by the individual herd owner and is designed to give the background of the use of B.A.I. strain 19 Brucella abortus vaccine.

Table 2. History of herds and tests performed.

Herds Nos. 1 and 2	
Year vaccination was begun	1942
Infection before vaccination	yes
Abortions before vaccination	yes
Abortions since vaccination	no
Age of calves at vaccination	6 to 8 months
Negative reactors to vaccination	yes, these animals are immediately disposed of
Were vaccinated animals from infected herds	yes
Unvaccinated animals in herd	no

Laboratory tests	
Tests performed :	Vaccinated
Milk serum agglutination test	negative
Agar slant suspension agglutination test	negative

The results indicate repeated attempts to prove the presence of the Brucella abortus organism.

Herd No. 1 was milked twice and contained 29 cows.

Herd No. 2 was milked once and contained 33 cows.

Table 3. History of herd and tests performed.

Herd No. 3	
Year vaccination was begun	1942
Infection before vaccination	yes
Abortions before vaccination	yes
Abortions since vaccination	very few
Age of calves at vaccination	4 to 8 months
Negative reactors to vaccination	The herd owner stated that his herd had experienced some instances where cows had calved 2 or 3 times then aborted. No blood tests were made to determine the blood titer reaction.
Were vaccinated animals from infected herds	No new animals introduced
Unvaccinated animals in herd	no

Laboratory tests

Tests performed	Vaccinated
Milk serum agglutination test	negative
Agar slant suspension agglutination test	negative

The results indicate repeated attempts to prove the presence of the Brucella abortus organism.

This herd was milked once and contained 20 cows.

Table 4. History of herd and tests performed.

Herd No. 4	
Year vaccination was begun	1946
Infection before vaccination	no
Abortions before vaccination	no
Abortions since vaccination	no
Age of calves at vaccination	6 to 10 months
Negative reactors to vaccination	none experienced
Were vaccinated animals from infected herds	no
Unvaccinated animals in herd	20 plus 1 bull

Laboratory tests

Tests performed	Vaccinated	Unvaccinated
Milk serum agglutination test	negative	negative
Agar slant suspension agglutination test	negative	negative

The results indicate repeated attempts to prove the presence of the Brucella abortus organism.

This herd was milked once and contained 26 cows.

Table 5. History of herd and tests performed.

Herd No. 5	
Year vaccination was begun	1942
Infection before vaccination	very little
Abortions before vaccination	some
Abortions since vaccination	yes
Age of calves at vaccination	4 to 8 months
Negative reactors to vaccination	yes, these are re-vaccinated
Evidence of infection from these animals	yes
Were vaccinated animals from infected herds	no additions to the herd have been made
Unvaccinated animals in herd	no

Laboratory tests

Tests performed	Vaccinated
Milk serum agglutination test	negative
Agar slant suspension agglutination test	negative

The results indicate repeated attempts to prove the presence of the Brucella abortus organism.

This herd was milked twice and contained 30 cows.

DISCUSSION

All tests on milk samples from the five herds were the same as used in preliminary tests, with the exception of the use of composite samples and swab utilization in the latter herds. In the preliminary examinations Br. abortus was isolated from the milk of old cows that had been vaccinated.

Five dairy herds were examined containing 118 vaccinated cows and 20 unvaccinated cows. No brucella organisms were found to be present in the milk of these animals.

Vaccination with B.A.I. strain 19 Br. abortus vaccine was practiced at ages of 6 to 8 months since 1942 in herds Nos. 1, 2, 3, and 5. Herd No. 4 began vaccination in 1946.

A small number of abortions had been experienced by herds 3 and 5 while herds 1, 2, and 4 had none after the vaccination program was begun.

The disposition of negative reactors varied. Immediate disposal was practiced in herds 1 and 2. No blood titer checks were made of animals in herd 3. Herd 4 experienced no reactors and herd 5 practiced revaccination of all negative reactors.

The history of the unvaccinated animals indicated their derivation from infection free herds.

Confidence was expressed by the herd owners in the results of the calfhood vaccination program due to the lowered to absent abortions in the tested animals.

CONCLUSIONS

Failure to prove the presence of Br. abortus organisms in the milk of the vaccinated animals may have been due to the administration of B.A.I. strain 19 Br. abortus vaccine at calfhood giving an increased resistance to the animals together with the prevention of infected entries into the herd.

The absence of Br. abortus organisms in the milk of unvaccinated animals can be accredited to freedom from infection in the herd.

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